Interleukin-1 polymorphisms are associated with the inflammatory response in human muscle to acute resistance exercise

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Inflammation appears to play an important role in the repair and regeneration of skeletal muscle after damage. We tested the hypothesis that the severity of the inflammatory response in muscle after an acute bout of resistance exercise is associated with single nucleotide polymorphisms (SNPs) previously shown to alter interleukin-1 (IL-1) activity. Using a double-blind prospective design, sedentary young men were screened ($n = 100$) for enrolment ($n = 24$) based upon having 1 of 4 haplotype patterns composed of five polymorphic sites in the IL-1 gene cluster: IL-1A (+4845), IL-1B (+3954), IL-1B (−511), IL-1B (−3737) and IL-1RN (+2018). Subjects performed a standard bout of resistance leg exercise and vastus lateralis biopsies were obtained pre-, and at 24, and 72 h post-exercise. Inflammatory marker mRNAs (IL-1β, IL-6 and tumor necrosis factor-α (TNF-α)) and the number of CD68$^+$ macrophages were quantified. Considerable variation was observed in the expression of these gene products between subjects. At 72 h post-exercise, IL-1β had increased in a number of subjects ($n = 10$) and decreased ($n = 4$) or did not change ($n = 10$) in others. Inflammatory responses were significantly associated with specific haplotype patterns and were also influenced by individual SNPs. Subjects with genotypes 1.1 at IL-1B (+3954) or 2.2 at IL-1B (−3737) had approximately a 2-fold higher median induction of several markers, but no increase in macrophages, suggesting that cytokine gene expression is elevated per macrophage. The IL-1RN (+2018) SNP maximized the response specifically within these groups and was associated with increased macrophage recruitment. This is the first report that IL-1 genotype is associated with the inflammation of skeletal muscle following acute resistance exercise that may potentially affect the adaptations to chronic resistance exercise.

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Intense physical activities, such as resistance exercise, with a strong eccentric component, cause micro-injury to skeletal muscle (Dolezal *et al.* 2000). The body senses the trauma and repair is initiated with the induction of a typical acute-phase response (MacIntyre *et al.* 1995; Tidball, 1995). Cytokines are thought to initiate and regulate this process and have been well characterized systemically (Pedersen & Toft, 2000). Among the first genes activated with almost any challenge to the body are the pro-inflammatory cytokines interleukin (IL)-1β and TNF-α, and with micro-injury to the muscle, quiescent resident macrophages begin to produce these mediators (Tidball, 1995). These cytokines trigger increased proteolysis and act as chemoattractants for an influx of macrophages from the circulation (Rappolee & Werb, 1992; Mansoor *et al.* 1996; Fielding & Evans, 1997; Wray *et al.* 2003). Macrophages phagocytize cellular debris and can continue secretion of IL-1β even

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5 days post-injury (Fielding *et al.* 1993). They are also a rich source of growth factors postulated to stimulate myogenesis (Robertson *et al.* 1993) that involves muscle satellite cell proliferation, migration to the site of injury, and fusion either with existing fibres for repair or with each other to replace degenerated muscle fibres (Vierck *et al.* 2000). Thus, inflammation may also be involved with the mechanisms of hypertrophy.

Inflammation must be strictly regulated or considerable secondary tissue damage and myopathy can ensue (Authier *et al.* 1997; Tidball, 2002). Infusion of IL-1β or TNF-α suppresses protein synthesis, induces skeletal muscle catabolism, and results in anorexia in rats (Ling *et al.* 1997). Increased expression of IL-6 has implicated cytokine-mediated events in reduced skeletal muscle function with age (Ferrucci *et al.* 1999; Barbieri *et al.* 2003), but IL-6 may actually possess anti-inflammatory properties within this context (Tilg *et al.* 1997). The finding that IL-1β stimulates IL-6 production in myoblasts (Gallucci *et al.* 1998; Pedersen *et al.* 2003) may also indicate that the coordinated regulation of cytokines is important for muscle repair and myogenesis. Indeed, both IL-6 and the IL-1 receptor antagonist (IL-1RA) increase dramatically in the plasma following strenuous exercise (Pedersen & Toft, 2000). Thus, there probably exists an optimal balance between inflammatory factors and their negative regulators that is most conducive to skeletal muscle health.

At least 90 genes or gene variations have been associated with differences in exercise performance and health-related fitness phenotypes (Perusse *et al.* 2003). Notably, single nucleotide polymorphisms (SNPs) in two genes, ciliary neurotrophic factor (Roth *et al.* 2001) and interleukin-6 (Roth *et al.* 2003), have been associated with strength and/or fat-free mass. Since much of the early injury response is shaped by the local release of IL-1β and TNF-α, we have investigated the influence of IL-1 gene variations among individuals on the muscle response to resistance exercise. The genes for IL-1 biological activity are found in a cluster on chromosome 2 and include the genes that encode the agonists IL-1α, IL-1β, and the antagonist IL-1RA. SNPs have been identified across the IL-1 gene cluster and certain SNPs in this region are in strong linkage disequilibrium (Cox *et al.* 1998) and define specific IL-1 haplotypes. Three haplotype groups are highly prevalent in Caucasian populations (Cox *et al.* 1998) and have been associated with altered levels of these proteins and other inflammatory markers (Hurme & Santtila, 1998; Shirodaria *et al.* 2000; Berger *et al.* 2002). As a consequence, these SNPs or closely linked SNPs, may influence the severity of the inflammatory response and are associated with disease (Buchs *et al.* 2001; Kornman & Duff, 2001). IL-1 gene variations have not been examined in relation to inflammatory myopathies or in the normal inflammatory response generated in skeletal muscle after a standard bout of resistance exercise which, if performed chronically, results in increased muscle size and strength. Given that genetic variations are known to influence the inflammatory response in other systems, we hypothesized that the magnitude of inflammatory marker induction in skeletal muscle after a standard bout of acute resistance exercise will be associated with specific IL-1 SNPs. The purpose of this research was to begin investigating the link between inflammation and genetics in relation to the normal metabolic response that leads to hypertrophy. Our results suggest that variations within the IL-1 gene cluster may be predictors of the adaptability of skeletal muscle, as the muscle inflammatory response in young men after weight-lifting was influenced by their IL-1 genotype.

Methods

Study design

The study was a double blind prospective evaluation of the influence of IL-1 gene polymorphisms on muscle response to exercise. Because of the difficulty of interpreting the biological influences of genetic differences in small populations, we used a genotype-specific clinical model. In this model subjects were pre-selected based on the prior determination of specific patterns of IL-1 SNPs that not only tagged common haplotypes but were also relevant to distinguishing functional differences among the haplotypes (data not shown). Subjects were enrolled based on fitting one of three specific patterns or a fourth 'uncertain' pattern (Table 1). Pattern assignments were performed by investigators who did not participate in any of the clinical or laboratory assessments. During the study, investigators and subjects were blinded to genotype.

Subjects

One-hundred healthy but sedentary (i.e. no structured physical activity for at least a year) non-smoking males were screened. Subjects were informed of the procedures and risks, completed a medical history questionnaire, and gave written consent in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the University of Arkansas for Medical Sciences. Subjects also provided a blood sample for genotype analysis. Twenty-four subjects were selected to complete the protocol based upon their IL-1 haplotype. The selected subjects had the following characteristics: age 30 ± 7 year (mean $\pm$ S.D.), weight 85.5 ± 17.4 kg, height 177.7 ± 7.1 cm, and body mass index 26.9 ± 4.3 kg m^{-2}. Subjects were required to refrain from analgesic and anti-inflammatory medications (e.g. ibuprofen and acetaminophen) or participating in any unusual physical activity and/or diet modifications 3 days prior and throughout the protocol.

Table 1. IL-1 genotype criteria for subject selection by haplotype group

	Pattern prevalence[2]	n[3]	IL-1A (+4845)	IL-1B (+3954)	IL-1B (−511)	IL-1B (−3737)
Pattern 1 haplotype tags[1]	5.2%		2	2	1	1
Genotypes preferred in selection of group		4	2.2	2.2 or 1.2	1.1 or 1.2	1.1 or 1.2
Genotype exceptions in group		1	2.2	1.1	1.2	1.1
		1	2.2	2.2	1.1	2.2
Pattern 2 haplotype tags[1]	3.1%		1	1	2	1
Genotypes preferred in selection of group		6	1.1 or 1.2	1.1	2.2	1.1
Genotype exceptions in group		0				
Pattern 3 haplotype tags[1]	4.3%		1	1	1	2
Genotypes preferred in selection of group		6	1.1 or 1.2	1.1	1.1	2.2
Genotype exceptions in group		0				
Uncertain pattern assignment		3	1.2	1.2	1.2	1.1
		2	1.1	1.1	1.2	1.2
		1	1.2	1.2	1.1	1.2

[1]Alleles that were used at the indicated loci to select subjects. Where possible subjects were selected who were homozygous at the haplotype tags. [2]The observed prevalence of the composite genotype pattern was calculated in a population of 765 Caucasian individuals. [3]Number of subjects selected with the indicated composite genotype for inclusion in the study.

Genotypic analysis

Genomic DNA was extracted from whole blood using the QIAamp DNA miniprep kit (Qiagen, Valencia, CA, USA). Single nucleotide polymorphisms were examined at five IL-1 loci: IL-1A (+4845, G/T), IL-1B (+3954, C/T), IL-1B (−511, C/T), IL-1B (−3737, C/T) and IL-1RN (+2018, T/C). The first nucleotide, the more common allele, is labelled as '1' and the second nucleotide, the polymorphism, is labelled '2'. Assays were performed using the Taqman 5′ nuclease assay (Di Giovine *et al.* 2000).

Exercise protocol

Following 10 min of light cycling for warm-up, each subject's one-repetition maximum (1-RM) was obtained for bilateral leg press (1522 ± 256 Nm), leg curl (244 ± 38 Nm) and leg extension (286 ± 40 Nm) (Keiser Corp., Fresno, CA, USA). Subjects rested for 5 min and then completed three sets of eight contractions followed by a fourth set to voluntary failure for each of the three exercises. Resistance was set at 80% of the subject's 1-RM. Subjects were given 2 min of rest between sets and 5 min between exercises. Subjects were allowed to walk and perform light stretching during the rest periods. Verbal encouragement was consistently given to all subjects during the resistance exercise. The purpose of the chosen exercise protocol was to mimic a standard resistance exercise session that is commonly performed chronically to increase muscle mass and strength.

Muscle biopsy

Muscle biopsy samples were taken from the vastus lateralis muscle just prior to the exercise session and 24 and 72 h post-exercise. The first biopsy was taken from the subject's non-dominant leg and the remaining biopsies were taken from the dominant leg. The 24 and 72 h biopsies were taken through separate incisions with the 72 h biopsy proximal to the 24 h biopsy. Tissue was obtained after local anaesthetic (lidocaine HCl 1%, 3 ml) with the use of a 5 mm Bergstrom needle with suction (Evans *et al.* 1982). The muscle was cleansed of excess blood, connective tissue and fat, and cut into two samples. One sample was frozen in liquid nitrogen for RNA isolation, and the other was vertically mounted in tragacanth gum (Sigma G-1128) and frozen in isopentane cooled to the temperature of liquid nitrogen. All samples were stored at −80°C until analysis.

RNA purification

Frozen tissue samples, ranging in weight from 25 to 116 mg, were powdered using a stainless-steel pulverizer and liquid N_2. Total RNA was isolated using the Totally RNA kit according to the supplied protocol (Ambion, Austin, TX, USA). The tissue was disrupted in a scaled-volume of lysis buffer using a high speed electric homogenizer. Upon procedure completion, RNA was resuspended in 50 μl of diethylpyrocarbonate (DEPC) water/0.1 mM EDTA and then further purified using the RNAqueous kit (Ambion). RNA was eluted in 100 μl of

Table 2. Real-time PCR primer sequences and assay characteristics

	Accession number	5′-forward primer-3′ and 5′-reverse primer-3′	Standard range[1]	Sample input[1]	Primer conc.[2]	Prod. T_m[3]	Pd T_m[3]	% E[4]
IL-1β	NM_000576	GCTTGGTGATGTCTGGTCCAT CACCACTTGTTGCTCCATATCCT	80–1.25	50	265	78.5	—	97
IL-6	M54984	TACATCCTCGACGGCATCTCA GCCTCTTTGCTGCTTTCACA	80–1.25	100	300	78.4	—	95
TNF-α	NM_000594	TCTGCCTGCTGCACTTTGG GCCAGAGGGCTGATTAGAGAGA	80–1.25	50	275	82.1	76.0	98
IL-1α	NM_000575	GTTTAAGCCAATCCATCACTGATG GACCTAGGCTTGATGATTTCTTCCT	80	—	250	79.9	—	—
18 S	ABI	TTCGGACGTCTGCCCTATCAA ATGGTAGGCACGGCGACTA	5–0.08	1	325	77.8	—	98

[1]Standard curve range and sample input units are ng RNA equivalent of cDNA. [2]Primer concentrations are in nM. [3]PCR product and primer dimer T_m units are °C. [4]%E is the amplification efficiency of the PCR assay.

DEPC water/0.1 mM EDTA and then treated with DNase using DNA-free reagents (Ambion). RNA integrity was confirmed by agarose-gel electrophoresis.

Real-time PCR

All aspects of real-time quantitative PCR were performed using the protocols, chemistries, and the amplification and detection systems of Applied Biosystems (Applied Biosystems, Foster City, CA, USA). For each sample, cDNA was synthesized with random hexamers and 2 μg of total RNA using components from the Taqman Reverse Transcription Reagents. The cDNA was aliquoted and stored at −80°C until use. PCR primer sequences were selected from the NCBI database using the Taqman Probe and Primer Design function (ignoring the probe) of the Primer Express v 1.5 software and were synthesized by Invitrogen (Table 2). Reactions were carried out using 2 × SYBR Green Master Mix and the ABI Prism 7700 Sequence Detection System. Assay parameters (Table 2) were optimized and relative gene expression was calculated using standard curves (log of the ng RNA-equivalents of cDNA *versus* cycle number) generated using 4-fold serial dilutions of cDNA. The cDNA was a pool of the two subjects with the highest expression levels for IL-1β and IL-6. Data were normalized to 18 S ribosomal RNA. Values presented (Table 4 and Fig. 1) are ng of each gene of interest per ng of 18 S relative to a standard curve for each gene of interest.

Immunohistochemistry

Serial 7 μm cryostat sections were cut from the biopsy material and stored at −20°C. Sections were rehydrated in PBS, blocked with PBS + 0.3% H_2O_2 and incubated with an anti-human CD68 macrophage monoclonal antibody (DAKO, Carpinteria, CA, USA; M0718, 1 : 100) for 1 h at room temperature. After washing 3 × 5 min in PBS, sections were incubated with biotin-labelled, human-absorbed, goat anti-mouse IgG (KPL,Gaithersburg, MA, USA; 1 : 400). After washes in PBS, sections were incubated in streptavidin peroxidase (Zymed, San Francisco, CA, USA) and visualized with the DAB peroxidase substrate kit (Vector Laboratories, Burlingame, CA, USA). Sections were post-fixed in PBS containing 4% paraformaldehyde for 15 min at 4°C. Sections were rinsed, dehydrated and then a cover slip applied. CD68 positive cells were quantified using a Nikon Eclipse E400 microscope equipped with a Sony DXC390P camera using OsteoMeasure v 4.1 software (Osteometrics, DeCatur, GA, USA). The number of $CD68^+$ cells per volume of the cross section (cells mm^{-3}) was determined.

Statistics

Mann-Whitney tests were used to detect differences between the median values between genotypic groups. Wilcoxon tests were performed to determine differences between the time points within groups. For individual SNP analyses and due to the small group size for certain genotypes, the heterozygous (1.2) subjects for each SNP were grouped with either the homozygous wild type (1.1) or homozygous polymorphic (2.2) subjects. Box plots were used to decide which groups displayed the most similar marker expression. Tree analysis was used to identify an association between IL-1 SNPs and has been described elsewhere (Curran *et al.* 1993; Schumacher *et al.* 1993). The algorithm used to make a tree identified the best way to split the variable into two groups and optimize the specific function. Groups were sought that were most different for marker expression based on 0, 1 or 2 of the different genotype groups. All polymorphic sites were considered for each split. Differences in mean induction were analysed using a two-tailed independent samples *t* test on the logarithms of the data under the assumption that larger sample sizes would produce equal variance. For presentation, non-log transformed means are shown (Fig. 4).

Results

Genotypes

To determine if certain IL-1 polymorphisms are associated with the inflammatory response in muscle to acute resistance exercise for young men, 100 subjects were genotyped for five SNPs that are highly informative of the linkage disequilibrium patterns across the gene cluster: IL-1A (+4845), IL-1B (+3954), IL-1B (−511), IL-1B (−3737) and IL-1RN (+2018). Subjects were selectively enrolled based upon composite genotypes that most closely matched to homozygous carriage of one of three haplotype patterns ($n = 6$ each, Table 1). Six subjects were also selected for a fourth 'uncertain' group whose genotypes were not clearly matched to a haplotype pattern. IL-1RN (+2018) genotype was not considered in the subject selection criteria; however, the frequency of the IL-1RN polymorphism as well as the frequencies of the other individual SNPs within this study population is shown in Table 3. These results represent the specific IL-1 genetic makeup of the 24 subjects who were accepted to complete the exercise protocol and inflammatory marker quantification.

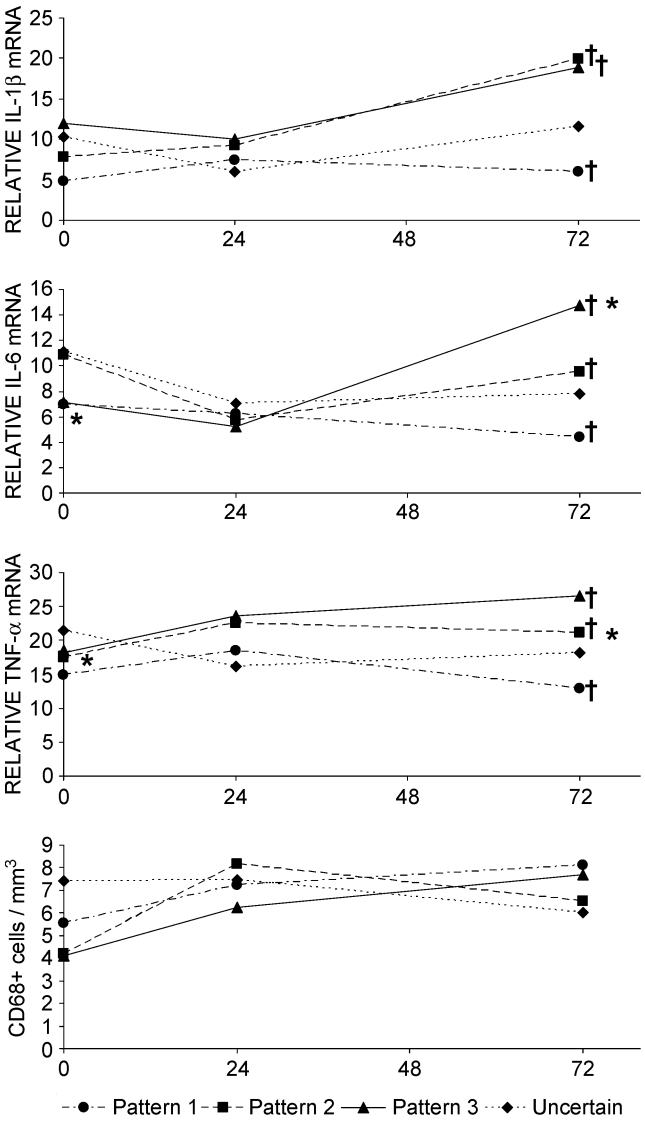

Figure 1. IL-1β, IL-6 and TNF-α mRNAs were measured from vastus lateralis biopsies pre-, 24 and 72 h after acute resistance exercise by quantitative real-time PCR
Data were normalized to 18 S ribosomal RNA. The number of $CD68^+$ cells (macrophages) ×100 was quantified using immunohistochemistry. Differences between median expression were significant ($P < 0.05$) by the Mann-Whitney test († between haplotype pattern groups 2 and 3 *versus* 1, * between pre-exercise and 72 h time points).

Inflammatory markers

Five markers of inflammation were quantified at the mRNA (IL-1α, IL-1β, IL-6 and TNF-α) or protein (macrophage-marker CD68) levels using vastus lateralis biopsies collected pre-, 24 and 72 h post-acute resistance exercise. Initially, the data were examined without regard to haplotype patterns. IL-1α mRNA proved to be extremely rare in muscle tissue and was only reliably detected at the highest template concentration of the standard curve; therefore, IL-1α was excluded from further analysis. For IL-1β, IL-6, TNF-α and CD68, subjects displayed considerable variation both in their pre-exercise levels

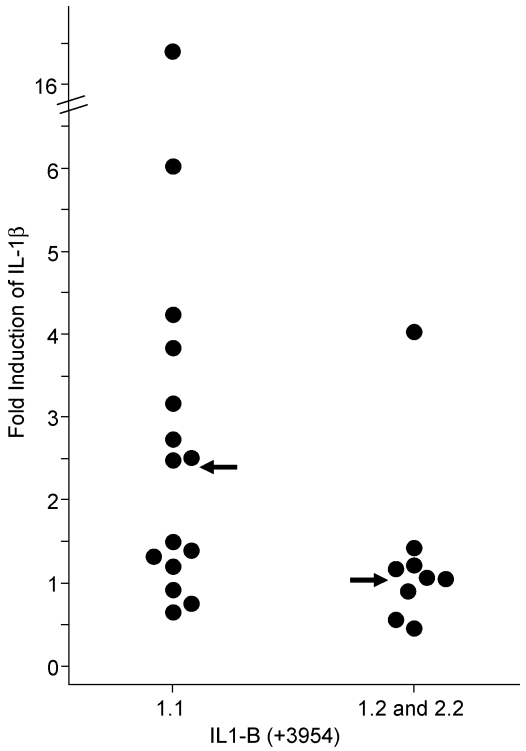

Figure 2. IL-1β mRNA was measured from vastus lateralis biopsies pre- and 72 h after acute resistance exercise
Data are presented as the 72 h/0 h measurement ratio or the fold-induction for each subject. The difference between medians (arrows) is significant ($P < 0.05$) by the Mann-Whitney test.

Table 3. Percentage of subjects possessing each IL-1 SNP for those screened and those (accepted)

SNP site	1.1	1.2	2.2
IL-1A (+4845)	48.0 (50.0)	42.0 (25.0)	10.0 (25.0)
IL-1B (+3954)	60.0 (62.5)	37.0 (29.2)	3.0 (8.3)
IL-1B (−511)	44.0 (41.7)	43.0 (33.3)	13.0 (25.0)
IL-1B (−3737)	32.0 (50.0)	45.0 (20.8)	23.0 (29.2)
IL-1RN (+2018)	58.0 (41.7)	36.0 (54.2)	6.0 (4.2)

Table 4. Inflammatory markers show a varied response between subjects before and after exercise

	Baseline range	Fold induction 72 h post-exercise (number of subjects)[3]		
		≤ 0.8	$0.8 < 1.5$	≥ 1.5
IL-1β	1.67–23.99[1]	4	10	10
IL-6	1.33–23.25[1]	8	9	7
TNF-α	6.14–30.55[1]	3	13	8
CD68	1.56–36.47[2]	6	9	9

Values represent [1]marker expression normalized to 18 S or [2]the number of $CD68^+$ cells $\times$ 100 per mm^3 of muscle section. [3]The between group cut-off values were determined by visual inspection of the data.

and for the post-exercise response (Table 4). Baseline levels showed from approximately a 5-fold range between subjects for TNF-α to a 23-fold range for $CD68^+$ macrophages. Post-exercise, subjects were segregated into distinct response groups based on the change in marker expression. For instance, when analysed as a ratio of the 72 h level to baseline, IL-1β mRNA increased ($n = 10$) for a number of subjects, and decreased ($n = 4$), or did not change ($n = 10$) for others. Subjects displayed the same variety of responses for IL-6, TNF-α and CD68. Further analysis was performed to determine if these distinct responses were associated with IL-1 genotype.

Inflammatory marker association with IL-1 polymorphisms

The levels of IL-1β, IL-6, TNF-α and CD68 at pre-, 24 and 72 h post-exercise were analysed with respect to the four haplotype groups (pattern 1, pattern 2, pattern 3 and uncertain) for differences between groups as well as differences over time using the Mann-Whitney test (Fig. 1). Median IL-1β, IL-6 and TNF-α mRNA levels were significantly greater for patterns 2 and 3 than for pattern 1 at 72 h post-exercise ($P < 0.05$). Furthermore, this 72 h expression was significantly greater than baseline with IL-6 for pattern 3 and TNF-α for pattern 2 ($P < 0.05$). Expression also tended to be higher at 72 h than baseline with IL-6 for pattern 2, TNF-α for pattern 3 and IL-1β for both, but the levels failed to reach significance. The number of $CD68^+$ macrophages was not significantly different between the haplotype patterns and did not significantly change after exercise within any pattern.

Table 5. Mann-Whitney comparison for median fold-induction of inflammatory markers 72 h post-acute resistance exercise

	IL-1β	IL-6	TNF-α	$CD68^+$
IL-1B (+3954)				
1.1	*2.48	*1.38	*1.43	1.34
1.2 and 2.2	1.07	0.68	0.87	0.98
IL-1B (−3737)				
1.1 and 1.2	1.32	0.84	1.16	1.06
2.2	1.50	*1.53	2.06	1.34

*$P < 0.05$.

The data were analysed to determine which individual SNPs within these haplotype patterns were most closely associated with pattern-specific inflammatory marker induction 72 h post-exercise. IL-1B (+3954) was associated with the largest differences in marker induction. Genotype 1.1 subjects showed significantly higher median fold-induction of IL-1β mRNA than those carrying allele 2, i.e. genotypes 1.2 and 2.2 ($P < 0.05$, Fig. 2). Induction was also greater for the 1.1 subjects for IL-6 and TNF-α, but CD68 did not reach significance (Table 5). The IL-1B (−3737) locus was also associated with differences in median marker induction (Table 5). IL-6 was significantly higher for subjects 2.2 than for those of 1.1 and 1.2 ($P < 0.05$). This trend did not reach significance for the other three markers. Analyses of the other three IL-1 sites showed no association between marker induction and genotype for IL-1A (+4845), IL-1B (−511) or IL-1RN (+2018).

Cooperative effects between IL-1 SNPs

Multivariate analysis was performed to determine if marker induction was influenced by any associations between the IL-1 polymorphisms in the different genes. A tree method of analysis first confirmed that subjects 1.1 for IL-1B (+3954) exhibited greater median induction of the inflammatory markers than 1.2 and 2.2 subjects, as evidenced by the fact that the algorithm split these two groups along different branches (Fig. 3). A second split occurred for the IL-B (+3954) 1.1 subjects, this time according to their genotype at IL-1RN (+2018). Of those 1.1 for IL-1B (+3954), the subjects that carried allele 2 (1.2 or 2.2) at IL-1RN (+2018) showed greater average induction of IL-6 than those that were genotype 1.1. Mean IL-1β, TNF-α and CD68 also behaved accordingly along each branch of the tree. The sample sizes were too small to use the tree method of analysis to identify SNPs interacting with IL-1B (−3737), the other SNP that was individually associated with higher induction of inflammatory mediators. However, statistical testing of the

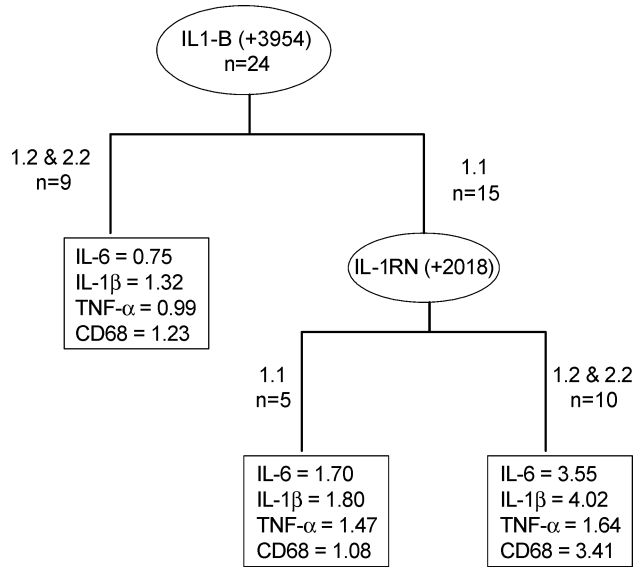

Figure 3. Analysis by a tree method of the association between inflammatory marker induction and IL-1 genotype
A decision-making algorithm was used to co-segregate subjects based on the fold-induction of each at 72 h post-exercise and their genotype for each polymorphic IL-1 site. The algorithm initially based the tree structure on the IL-6 response. Other marker quantities were added retrospectively for comparison.

individual subgroups showed that subjects 2.2 at IL-1B (−3737), who also carried allele 2 at IL-1RN (+2018), exhibited greater induction of all four markers (IL-6, IL-1β, TNF-α and CD68) than the subjects 2.2 for IL-1B (−3737) that lacked the polymorphism (Fig. 4). It should be noted that in this small sample there were no subjects who were both IL-1B (−3737) 2.2 and IL-1RN (+2018) 2.2. No other interactions between SNPs were observed.

Discussion

Association between IL-1 genotype and inflammatory response

The main finding of this investigation was that the IL-1 genotype influenced the inflammatory response in skeletal muscle after a standard bout of resistance exercise in young men. This work was motivated by reports that IL-1 SNPs are known to influence the magnitude of inflammation under other physiological circumstances. We hypothesized that these IL-1 SNPs might also ultimately be identified as determinants in skeletal muscle repair and hypertrophy. Specifically, we examined haplotype groups composed of polymorphisms in the IL-1A, IL-1B and IL-1RN genes with respect to the changes in inflammatory markers IL-1β, IL-6, TNF-α and CD68$^+$ macrophage number after weight-lifting. Since it would require large numbers of subjects to interpret biological influences of many potential combinations of haplotypes, subjects were pre-selected to have a limited set of haplotypes that are commonly found in Caucasians and have SNP tags that have been associated with IL-1 biological activity.

Subjects with specific IL-1 haplotypes (labelled patterns 2 and 3 in this study) exhibited significantly greater induction of inflammatory markers 72 h post-exercise than subjects with a different set of IL-1 gene variations. These results were confirmed by analysis of these data *versus* the individual IL-1 SNPs. Significantly greater marker induction was seen for two SNPs that are tags for the haplotype patterns 2 and 3. Subjects who were homozygous at either of two loci, IL-1B (+3954) 1.1 or IL-1B (−3737) 2.2 displayed significantly greater marker induction. Additionally, if an individual from either of those two groups also carried the IL-1RN (+2018) polymorphism (i.e. 1.2 or 2.2), the greatest inflammatory response was achieved. As no significant change in the number of CD68$^+$ cells was observed between haplotype patterns, the increased expression of cytokines by individuals with patterns 2 and 3 appears to be due to increased production per cell (presumably per macrophage but production by skeletal muscle cells was not ruled out) and not merely to a greater number of macrophages present. However, the greatest inflammatory response and significant changes in CD68$^+$ cell numbers were associated with the IL-1RN polymorphism, suggesting that heightened macrophage recruitment may contribute in these individuals. The coordinate regulation of IL-1β, IL-6 and TNF-α mRNAs and the increase in the number of CD68$^+$ cells indicate that an acute-phase response occurs in skeletal muscle after resistance exercise and that the response is influenced

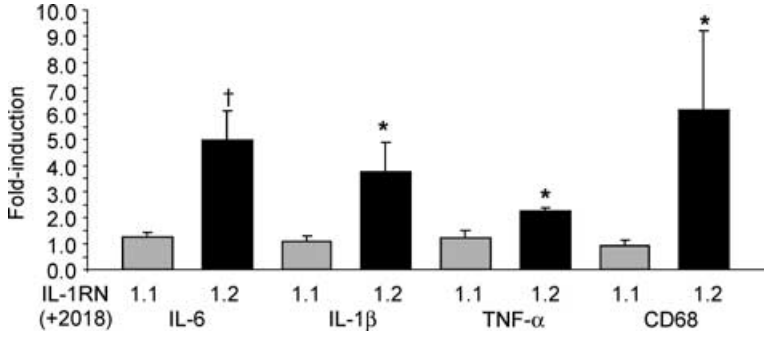

Figure 4. Inflammatory marker induction was compared within the IL-1B (−3737) 2.2 subject group according to IL-1RN (+2018)
Grey bars represent IL-1RN (+2018) 1.1 subjects ($n = 4$), and black bars represent the 1.2 subjects ($n = 3$). Significant differences were identified using an independent samples *t* test for equal variance. *P* values were † < 0.005 and * < 0.05.

by IL-1 gene variations that are commonly found in the population.

The IL-1 gene variations studied here have been previously examined for association with various chronic inflammatory diseases and with altered levels of inflammatory mediators. The alleles that are characteristic of haplotype pattern 1, including allele 2 at IL-1B (+3954), have been associated with more severe periodontal disease and cardiovascular disease events (Kornman & Duff, 2001), as well as increased monocyte production of IL-1β (Buchs *et al.* 2001; McDevitt *et al.* 2003). Pattern 1 alleles have also been associated with higher inflammatory mediators in gingival tissues (Shirodaria *et al.* 2000). In contrast, our results show that pattern 1 subjects displayed less induction of inflammatory mediators in skeletal muscle stimulated by resistance exercise. Haplotype patterns 2 and 3 were associated with a stronger inflammatory response in our model. The alleles that are characteristic of pattern 2, including allele 2 at IL-1B (−511), have been previously associated with coronary artery stenosis (Francis *et al.* 1999). Haplotype group 3 contains allele 2 of IL-1B (−3737), a newly identified SNP that influences IL-1B promoter activity (H. Chen, unpublished data). These results suggest that IL-1 genotype influences the normal physiological response to resistance exercise in healthy individuals and is different to the influence on inflammatory pathologies.

In addition to the identification of IL-1 haplotype patterns 2 and 3 as pro-inflammatory, an additive effect was seen in their association with the IL-1 receptor antagonist gene. Higher inflammatory marker induction was seen for subjects 1.1 at IL-1B (+3954) or 2.2 at IL-1B (−3737) and within these groups, the highest induction was seen for those that also possessed the IL-1RN polymorphism. Studies have found that the IL-1RN polymorphism can be associated with increased or decreased IL-1RA production (Hurme & Santtila, 1998; Tountas *et al.* 1999). Increased IL-1RA in skeletal muscle would require elevated IL-1β, as was observed in +3954 1.1 and −3737 2.2 individuals, to overcome the increased antagonism and exert a biological effect. Conversely, decreased production of IL-1RA would hinder regulation of the inflammatory response. These findings, together with those in the literature, suggest that the inflammatory response is influenced by IL-1 haplotype in a tissue-specific manner and is dependent upon the context of the stimulation.

Role of the inflammatory response in skeletal muscle adaptability

The idea that cytokine levels in muscle are related to muscle adaptability to exercise is supported by studies examining TNF-α and resistance training (Greiwe *et al.* 2001). TNF-α is elevated in skeletal muscle of the elderly, but decreased with resistance training. Further, an inverse correlation exists between TNF-α receptor abundance and strength gains after training (Bruunsgaard *et al.* 2004). The high degree of variability observed in the 24 healthy young men studied here suggests that the inflammatory response may contribute to normal differences in the adaptive response to resistance exercise training that exists between individuals. A study using a reverse cycling model of damaging exercise also showed that immunological changes in muscle were highly variable (Malm *et al.* 2000). Although it is possible that some variability may be due to heterogeneity within the muscle such that the biopsy does not accurately assess the inflammatory state of whole muscle, our results suggest that the variability in the muscle inflammatory response to exercise can be accounted for by differences in IL-1 haplotype.

The associations between IL-1 gene variations and the acute inflammatory response to resistance exercise reported here suggest that cytokine function in muscle ageing, wasting, injury and hypertrophy is, at least partially, genetically regulated. Although IL-1 and the inflammatory response appear to be required to initiate recovery from resistance exercise, this response may need to be limited for optimal muscle adaptation to ensue. Having identified IL-1 gene variations that influence the response of muscle tissue to an exercise stimulus, we are now testing whether specific IL-1 haplotypes are beneficial or detrimental for muscle repair and the adaptability to resistance training.

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